UNIVERSITY OF GONDER

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOLOGY, MS.c IN APPLIED MICROBIOLOGY

Assessment of the role of *Pseudomonas fluorescence L* on growth promoting of Maize (*Zea mays L*), wheat (*Triticum aestivum L*) and Sorghum (*Sorghum bicolor L*)

By: Yonas Tassew

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A Thesis submitted to Department of Biology, College of Natural and Computational Sciences, University of Gondar in partial fulfillment of the requirements for the degree of Master of Science in Biology (Applied Microbiology)

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LISTS OF ABBREVIATIONS

ACC=1- AminoCyclopropane-1-Carboxylate

CFU=Colony Forming Units

IAA=Indole Acetic Acid

KMB=king's medium B

MHC=Moisture Holding Capacity

NA=Nutrient Agar

PGPR= Plant Growth Promoting Rhizobacteria

Rpm = Revolution per Minutes

SPSS=Statistical Products and Service Solutions

UV = Ultraviolet

ABSTRACT

The use of plant growth promoting rhizobacteria has been proven to be an environmentally sound way of increasing plant growth. *Pseudomonas fluorescence* is an important group of Plant growth promoting rhizobacteria and the most abundant microorganism in the rhizosphare. The purpose of this study was to assess the role of *Pseudomonas fluorescence* on growth promoting of maize, wheat and sorghum. The study design was complete random design based on laboratory and pot experiment. The study was conducted at University of Gondar, by collecting rhizosphare soil samples from fields of growing of cereal crops which are found at around Debark. Three isolates were effectively identified as *Pseudomonas fluorescence* strains. Mixtures of *Pseudomonas fluorescence* strains showed significant increases in all the parameters (shoot height, shoot fresh weight, shoot dry weight, root length and root dry weight) measured. The isolates induced inhibition zones ranging from 10-29mm towards the *Fusarium oxysporium*. The highest germination percentage (91.66%) was observed with the seeds inoculated with the combination P.f1, P.f2 and P.f3. In pot experiment, it was observed that inoculation with *Pseudomonas fluorescence* strains combinations significantly increased overall growths of maize, wheat and sorghum. The highest increased shoot height of those cereal crops achieved by treatment one (T1), which increased shoot height by 24.33cm and 17.67cm for maize and wheat respectively and also for sorghum 15.00cm achieved by treatment one (T1) and two (T2). Combination of introductions of *Pseudomonas fluorescence* isolates is very effective in promoting growth than single isolates. Inoculation of *Pseudomonas fluorescence* strains as bio fertilizer will be useful in increasing cereal productivity and contribute to tackling food deficits in Ethiopia. There is a very limited knowledge of the use of PGPR in agriculture in Ethiopia. The current study therefore contributes for utilizing plant-associated bacteria to develop productivity of organic farming and sustainability in Ethiopia.

Key words: *Pseudomonas, Fusarium oxysporium,* Germination, Rhizosphare, Plant growth promoting rhizobacteria

1. INTRODUCTION

The utilization of synthetic composts and pesticides has caused harm to the environment. These agents are both dangerous to humans and animals, and may persist and accumulate in natural ecosystem and a response to this issue is supplanting chemicals with organic methodologies, which are viewed as more environment friendly in the long haul (Suman *et al.,* 2016). One of the rising examination territories for the control of various phytopathogenic specialists is the utilization of plant growth promoting rhizobaceria, which are capable of preventing the phytopathogen from harm. *Pseudomonas fluorescence* representing a group of PGPR can advancement development and suppress plant pathogens by various systems (Nihorimbere *et al.,* 2011).

Plant growth promoting rhizobacteria can exhibit a variety of characteristics responsible for influencing plant growth. An effective PGPR should have at least three characters of promoting plant activities which are phytostimulator, root colonization competency and bio control agent against plant pathogens (Bloemberg *et al.,* 2001). PGPR colonize the plant rhizosphere or inside the plant body (as endophytes) and promote plant growth by providing fundamental nutrients to plants (Kaur *et al.,* 2016). Most of the microbes that colonize the rhizosphere include bacteria, fungi, acticomycetes, protozoa, and algae. However, bacteria are the most abundant microbial present in the rhizosphere. Some genera of bacteria have been determined as PGPR including *Pseudomonas, Bacillus, Azospirillum, Azotobacter,* and *Bradyrhizobium* (Wahyudi *et al.,*2011). Since many species and strains of bacteria residing in rhizosphere have been shown to possess plant growth promoting traits they are collectively designated as PGPR. Bacteria promote plant growth in three different ways: synthesizing growth promoting hormones for the plants, facilitating the uptake of nutrients from the soil, and lessening or preventing the plants from diseases. The function of these microbial populations is well known and proven by their role in improving plant growth (Vejan *et al.,* 2016).

Plant growth promoting rhizobacteria were firstly proposed by Kloepper *et al.* (1980) and used mainly for the *Pseudomonas fluorescence* involved in biological control of pathogens and the enhancement of plant growth. Later, Kapulnik *et al.* (1981) extended to rhizobacteria capable of promoting directly the plant growth. Nowadays, this expression is used to refer to all bacteria living in the rhizosphere, improving plant growth by one or several methods (Haghighi *et al.,* 2011).

The rhizosphere, fraction of soil rich in exudates, is occupied by various bacterial groups named rhizobacteria. The soil around the rhizosphere is characterized by greater microbiological activity than the soil away from plant roots. Therefore, Soil adhered to root is defined as the rhizosphere (Singh, 2015). Plant root exudates attract microbes and feed them and, in turn, the plants often benefits from the microbes. Soil contains a wide diversity of microbes which are concentrated in nutrient abundant soil regions, including the topsoil layer and the region around the plant root. Hence, Plants can benefit from soil microbes in many ways. Certain microbes stimulate plant growth, enrich soils, degrade pollutants, or protect plants against pathogens (Karnwal, 2012).

Among rhizosphere habitants, strains of bacteria from genus *Pseudomonas* are generally recognized as PGPR. *Pseudomonas fluorescence* are often considered as predominant bacterium in rhizosphere and certain *Pseudomonas fluorescence* strains have received particular attention as potent bio fertilizing and bio control agents (Weller, 2007). *Pseudomonas fluorescence* is particularly suitable to be used as agricultural bio control agent. Because it can produce large amounts of secondary metabolites to protect plants from phytopathogens and stimulate plant growth (Alemu, 2014).

The application of *Pseudomonas fluorescence* has been extended to remediate contaminated soils in association with plants (Zhuang *et al.,* 2007). This review covers the perspective of soil-beneficial bacterium *Pseudomonas fluorescence* and the role it is playing in plant growth promotion via direct and indirect mechanisms. The further elucidation of different mechanisms involved will help to make these bacteria a valuable partner in future agriculture (Hayat *et al.,* 2010). Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. Therefore, PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms (Saharan and Nehra, 2011). Presence of *Pseudomonas fluorescence* inoculants in combination with microbial fertilizers play an effective role in stimulating plant yield and growth (Anitha and Kumudini, 2014). There is little correlation between the bacterial capacity for antibiosis activity on agar media and the ability of these organisms to biologically control pests in the field. Therefore, direct application of rhizobacteria to agricultural seeds has been highly recommended for the assessment of their potential as growth promoters (Schroth *et al.,* 1981).

Plant growth promoting rhizobacteria are being important components of organic farming that play vital role in maintaining soil fertility and sustainability. Although there is growing interest in replacing agrochemicals with bacterial inoculants (Mayak *et al.,* 2001), there have been few laboratory or field studies of the potential role of PGPR as plant growth promoting agents in Ethiopia. Therefore, there is a gap of not fully understood the mechanism by which PGPR promote plant growth. Now a day there is a gap of ten million tones of plant nutrients between removal of agricultural crops and supply through synthetic fertilizers. In the context of the environmental impact and cost of chemical fertilizers, excessive reliance on the synthetic fertilizers is not viable approach in long run because of the cost, both in domestic resources and foreign exchange, involved in setting up of fertilizer crops and sustaining the production. Therefore, using bio fertilizers/ PGPR would be the best option for farmers to increase productivity and also sustain their productivity (Mahdi *et al.,* 2010).

Scientific interest is related to PGPR potentiality in agriculture which is gradually increased as it offers an attractive way to replace the use of synthetic fertilizers, pesticides and other supplements. Recent progress in our understanding on the variety of PGPR in the rhizosphere along with their colonization ability and system of action should facilitate their application as a reliable component in the management of sustainable agricultural system. Resource poor farmers in the tropics especially in Ethiopia are not able to use sufficient amount of inorganic N fertilizer for crop production due to high cost, and hence looking for alternative means of improving available nitrogen in the soil is important. Therefore, more researches should be done on PGPR and their activities. Hence, more study and better understanding of the role of PGPR help in better use of PGPR in crop production in developing regions and the knowledge may have universal functions in all over the regions of the world-developing and developed like Ethiopia. It is important to realize the useful aspects of bio fertilizers/PGPR and implement its application to current agricultural practices. Ethiopia is a country of farmers and 85% of its population is engaged in farming activities. Therefore, the scope of organic farming or the use of bio fertilizer is bright in this country. Organic farming / bio fertilizers is the way towards sustainable development for a developing country like Ethiopia (Menamo *et al.*, 2015).

2. LITRETURE REVIEW

2.1. Plant Growth Promoting Rhizobacteria

The beneficial free-living soil bacteria that exist in association with the roots of different plants which favorably have an effect on the plant growth and product of commercially important crops are generally referred to as PGPR. There are two types of bacteria that are beneficial to plants, one that can form a symbiotic relationship with the plant, which involves formation of specialized structures or nodules on host plant roots and bacteria that are free-living and interact with the roots in the soil (Glick, 2005).

Plant growth promoting rhizobacteria colonizing the surface or inner part of roots play an important positive role that directly or indirectly influences plant growth and development. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; Suppression of plant disease, improved nutrient acquisition, phytohormone production (Sureshbabu *et al.*, 2016).

Beneficial effects of microorganisms have often been evaluated based on faster seed germination, better seedling emergence, and increased plant growth (Karnwal, 2012). Many reports have evaluated the *Pseudomonas fluorescence* as growth promoting rhizobacteria and /or biological control agent. Experiments carried out in laboratory, growth chamber and field condition evaluate the effect of PGPR and bioprotecting rhizobacteria on seed pathogens, seed germination, plant growth, and grain yield of wheat (Luz, 2001).

Pseudomonas fluorescence stimulates growth directly as it can improve the supply of nutrients, such as nitrogen and phosphorous or by production of phyto hormones like auxin, cytokinins and gibberellins (Suresh *et al.*, 2010) and also Production of growth hormones like indole acetic acid (IAA), phosphate- solubilization and uptake of iron (Sharma and Johri, 2003). Indirectly, PGPR promote development by the suppression of pathogens mediated by different mechanisms (Botelho and Mendonça-hagler, 2006). These indirect mechanisms of plant growth promotion by PGPR include: depletion of iron from the rhizosphere, synthesis of antibiotic production, depletion of production of fungal cell wall lysing enzymes, antifungal metabolites and induced systemic resistance. The competition for sites on roots and production of phytohormones have also suggested to be one of the mechanisms by which PGPR stimulate plant growth (Shurti *et al.*, 2013).

Plant growth promoting rhizobacteria can be classified depending on their inherent activities as: bio fertilizers (capable of accelerating the accessibility of nutrients to plant), phytostimulators (capable of facilitating the plant growth usually by synthesizing phytohormones), rhizoremediators (involved in the degradation of organic pollutants) and biopescticides (capable of managing plant diseases by the production of antimicrobial metabolites) (Khan *et al.,* 2009).

Pseudomonas fluorescence strains had the ability to improve sorghum (*Sorghum bicolor L.*) yield and yield components including height, number of leaves, fresh footage yield and dry forage yield. The effect of *Pseudomonas* significantly affected maize (*Zea mays L.*) growth and yield, iron up take and chlorophyll content; increasing plant iron content from 91.02 to 110.16 mg/kg and plant biomass from 49.50 to 79.25g/pot. Inoculation of wheat seed with *Pseudomonas fluorescence* resulted in the enhancement of wheat (*Triticum aestivum L.*) yield, height, tiller number, biomass and grain yield (Chegini *et al.,* 2015). Seed bacterization of sorghum with *Pseudomonas fluorescence* enhanced the uptake of essential macro and micro-nutrients resulting in overall increase of plant growth (Praveen *et al.,* 2012).

The bacteria genera of *Pseudomonas fluorescence* is widely used (Pongdet, 2010) and is one of the rhizobacteria groups that have an important role in plant growth promoter and plant health (Wahyudi *et al*., 2011). Isolation of rhizobacteria with plant-growth promoting properties represent the crucial steps for creation of compatible bacterial mixture to increase bio control performances of microbial inoculums for agriculture field applications (Djuric *et al.,* 2011). The direct use of microorganisms to control plant pests, to promote plant growth is an area of rapidly expanding research. In the last decades studies on PGPR have been increasing at an ever increasing rate since the expression was first used by Kloepper and coworkers in the late 1970s. At present, the use of biological system is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant management system (Sengupta and Gunri, 2015).

Maize (*Zea mays L.*) is one of the most important food crops which is the third rank after wheat (*Triticum aestivum L.*), and rice. And that of Sorghum (*Sorghum bicolor L.*), is an important fifth largest cereal crops in the world (Praveen *et al.,* 2012). But they are subject to many soil borne plant pathogens that reduce grain production in many parts of the world. Sorghum is a dry land summer cereal which is staple crop in arid and semi-arid areas in Ethiopia. It is also a staple food for more than 500 million people in more than 30 countries,

although maize has often replaced its use. In Ethiopia, where traditional agriculture predominates, the average yield is very low, ranging between 200 and 1500 Kg/ha compared to developed countries such as the USA where commercial yields were 3775- 4400Kg/ha in the 1980s (Idris *et al.,* 2009).

The inoculation of PGPR, in tandem with the addition of inorganic nitrogen fertilizer, results in an increase in crop yields comparable or greater than that observed when conventional quantities of inorganic nitrogen are added. A research on wheat demonstrated maximum increases in yields of grain were observed in treatments where PGPR were used in combination with recommended dosages of inorganic fertilizer. A further study indicated that PGPR which demonstrated 1-aminocyclopropane-1- carboxylate-deaminase activity, such as *Pseudomonas fluorescence* could improve wheat yield and reduce the dependence on inorganic nitrogen by 25%, whilst giving an increase in wheat grain yield of 96% (Cummings, 2009).

Pseudomonas fluorescence commonly isolated from the rhizosphere has been shown to be ideal biological control agents as a result of ; many of the secondary metabolites from *Pseudomonas* inhibit other microorganisms, it is selectively stimulated in the rhizosphere and when introduced via seed coating it can dominate the rhizosphere population, a higher proportion of *Pseudomonas* is inhibitory to a variety of microorganisms in comparison with other soil bacteria, and they are extensively colonize the endorhizosphere (Stockwell *et al.,* 2011). A combination of introductions of *Pseudomonas fluorescence* isolates is very effective in controlling pests and diseases. The effectiveness of this particular combination is that these isolates do not compete for space and together colonize the root surface more effectively than single isolates (Hol *et al.,* 2013).

2.1.1. Taxonomic status of *Pseudomonas fluorescence*

The genus *Pseudomonas* belongs to the γ subclass of the proteobacteria, order Pseudomonales and family Pseudomonadaceae and genus *Pseudomonas*. *P. Aeruginosa, P. Chlororaphis, P. Fluorescens, P.pertucinogena, P. Putida, P. Stutzeri, P. Syringae and P. incertae sedis* (Botelho and Mendonça-hagler, 2006). This topic specifically will focus on *Pseudomonas fluorescence* because it is present in many environments, especially in the plant rhizosphere. *Pseudomonas fluorescence* encompasses a group of common, nonpathogenic saprophytes that colonize soil and plant surface environments. It is a common gram negative, rod shaped bacterium. As its name implies, it secretes soluble greenish fluorescent pigment

called fluorescein, particularly under conditions of low iron availability. It is motile by means of multiple polar flagella (Ganeshan and Kumar, 2005).

2.1.2. Occurrence of *Pseudomonas fluorescence* in rhizosphere

Pseudomonas species is a ubiquitous bacterium in agriculture soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been *Pseudomonas fluorescence* species. Considerable study is underway worldwide to exploit the potential of one group of bacteria that belong to *Pseudomonas fluorescence* (Sivasakthi *et al.,* 2014).

Microorganisms that colonize the rhizosphere can be classified according to their special effects on plants and the way they interact with roots, some being pathogens whereas other trigger beneficial effects. Rhizobacteria inhibit plant roots and apply a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR (Saharan and Nehra, 2011). The rhizosphere occurrence and activities of *Pseudomonas fluorescence*, as PGPR, have been considered as an important component of sustainable agriculture due to their plant growth promoting capacity as well as their bio control potential against phytopathogens. The ubiquitous occurrence and activities of *Pseudomonas fluorescence* in the rhizosphere of many crop plants have already been reported (Beattie *et al.,* 1995).

2.1.3. *Pseudomonas fluorescence* as PGPR

Plant growth promoting rhizobacterium plays an essential role in improving plant growth through a wide variety of modes. The mode of action of PGPR that promotes plant growth includes: a biotic stress tolerance in plants, nutrient fixation for easy uptake by plants, plant growth regulators, production of siderophores, the production of volatile organic compounds and the production of protection enzyme such as glucanase, ACC-deaminase for the prevention of plant diseases, and chitinase (Garcia *et al.,*2015).

The plant growth promoting rhizobacteria significantly affect the development and yield of different crops. A novel approach could be that composted material may be converted into a value added product such as an effective bio fertilizer by blending with PGPR which are free living soil bacteria that can either directly or indirectly assist growth of plants (Glick *et al.,* 1995) and rooting (Mayak *et al.,* 1999).

Many studies involve this bacterium that are able to improve plant growth and plant health and are implicated in the natural suppressiveness of certain soils to many soil-borne diseases. Inoculating wheat seeds with *Pseudomonas fluorescence* increased wheat yield by 147% in sterile soil and by 27% in natural soils. In another experiment, application of some *Pseudomonas fluorescence* strains increased wheat yield by 17% (Weller and Cook, 1983). *Pseudomonas fluorescence* can suppress soil-borne plant pathogens by a variety of mechanisms, such as, competition for iron, synthesis of antibiotics and bio surfactants, production of cell wall-degrading proteins and elicitation of induced systemic resistance (ISR) in the host plant against the phytopathogens (Chitra, 2015).

2.1.4. Role of *Pseudomonas fluorescence* in bio control aspect

Major advantage of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Ashrafuzzaman *et al.,* 2009). *Pseudomonas fluorescence* species possess an important place as biological control agents. They process an extensive variety for antifungal metabolites, which ensure those plants against phytopathogens. *Fusarium oxysporium* causes foot and root rot in tomato plants and is a serious problem for both field and greenhouse crops. One of the strategies to control *Fusarium* wilt is the use of antagonistic, root-colonizing *Pseudomonas* species. It has been demonstrated that different strains of *Pseudomonas fluorescence* suppress disease by different mechanisms. Therefore, application of a mixture of these bio control strains represents a viable control strategy (De Boer *et al.,* 1999). The production of a variety of antibiotics is an essential characteristic of *Pseudomonas fluorescence* (Fouzia *et al.,* 2015). Many of such antibiotics produced have a broad spectrum activity against phytophatogen but strain to strain variation do exist (Raaijmakers *et al.,* 2002).

Plant growth promoting rhizobacteria indirectly assist in plant development by suppression of deleterious microorganisms that inhibit plant growth or root pathogens through parasitism, antibiosis competition for nutrients and space within the surrounding area of plant roots, and/or activation of host defense responses. Suppression of deleterious microorganisms by PGPR is mainly by competing for available nutrients, by parasitism, production of proteins or toxins and inducing resistance by activating plant defense response against pathogens (Podile and Kishore, 2007). *Pseudomonas fluorescence* is known to suppress soil born fungal pathogens by producing antifungal metabolites and by rendering it unavailable to other organisms, sequestering iron in rhizosphere through the release of iron-chelating

siderophores. *Fusarium* wilts of various plant species controlled by this bacterium (Lemanceau and Alabouvette, 1993). *Pseudomonas fluorescence* is also establish themselves on plant roots and sink the available nutrients, thus limiting the available nutrients required for the growth of pathogen (Walsh *et al.,* 2001). Also compete with for nutrients with native rhizosphere microbes for removal of pathogen (Hayat *et al.,* 2012).

Pseudomonas possesses many traits that make it well suited as bio control and growth - promoting agents. These include the ability to grow rapidly in vitro and to be mass produced, rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere, produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores and growth substances), adapt to environmental stresses and compete aggressively with other microorganisms. In addition, *Pseudomonas* are responsible for the natural suppressiveness of some soils to soil borne pathogens (Weller *et al.,* 2002).

2.3. Beneficial and Harmful aspects of PGPR

The impact of PGPR generally on plant growth and health may be classified as neutral, deleterious or beneficial (Glick, 2005). Beneficial free living bacteria referred to as PGPR (Shruti *et al.,* 2013). Beneficial bacteria can be a significant component in the management of soil environment so as to achieve attainable crops yields. PGPR function through production of plant hormones such as auxins, ethylene, cytokinins, gibberellins and abscisic acid. Production of IAA, the compounds belonging to auxins, has been reported for several bacterial genera. A few PGPR function as a sink for ACC, quick for runner of precursor of ethylene in higher plants, by hydrolyzing it into α- ketobutyrate and ammonia, and in this way promote root growth by lowering indigenous ethylene levels in the micro-rhizo environment (Hayat *et al.,* 2010).

It is unquestionable that rhizobacteria play a crucial role in maintaining soil fertility and up grading plant growth and development. This growth betterment takes place with the help of several mechanisms although the reverse is true in some other studies (Saharan and Nehra, 2011). For example, the production of cyanide is known to be a characteristic of certain *Pseudomonas* species. Here, cyanide production by the *Pseudomonas* species bacteria is considered as a growth promotion as well as a growth inhibition characteristic. Moreover, cyanide acts as a bio control agents against certain plant pathogens (Martinez *et al.,* 2010). On the other hand, it can also cause adverse effects on plant growth. It is important to note that the effectiveness of auxin relies upon its concentration. For instance, at low

concentrations, it improves plant growth, whereas at a high level it inhibits root growth (Vacheron *et al.,* 2013).

Deleterious rhizobacteria are presumed to adversely affect plant development and growth through the production of undesirable metabolites (phytotoxins) or through competition for nutrients or inhibition of the other beneficial effects (Sturz and Christie, 2003). In order to exert their effect on plants, PGPR must be able to colonize the root, survive and multiply in the rhizosphere, facilitate plant growth (Barea *et al.,* 2005). Although PGPR are very effective at promoting plant development and growth, a few bacterial species may inhibit growth. However, this negative impact might just happen under certain specific conditions and also by some particular character. Thus, the selection of a particular strain is of the most importance in obtaining maximum benefits in terms of improved plant development (Vejan *et al.,* 2016).

The current agriculture faces new challenges, like degradation of soil, diminishing biodiversity, decline in productivity and increase in environmental contamination. This has led to think another option to the chemical agricultural method and hence organic Agriculture is gaining momentum currently (Reddy, 2014). PGPR being important components of organic farming play vital role in maintaining soil fertility and sustainability by fixing nitrogen , convert insoluble Phosphorus in the soil into forms of available to plants, thereby increases their efficiency and availability (Mahdi *et al.,* 2010).

In the contemporary agricultural practices millions of tons of fertilizers and pesticides are frequently but indiscriminately used to achieve optimum crop yields. Such artificial chemicals are however, not completely used up by plants and hence, persists in different forms in soil. The excessive use of fertilizer and pesticides are however, posing serious threat to the environments. Therefore, the sustainability in agricultural systems without compromising the environmental quality and conservation has become one of the major concerns of the scientists working in different agronomic area around the world. Discovery of plant growth promoting rhizobacteria Kloepper *et al.* (1980) has provided some relief to the poor agronomic practitioners largely due to low cost and easy and abundant availability. Currently attention has been paid to PGPR to replace agrochemicals (fertilizers and pesticides) for organic farming to promote growth by a variety of mechanisms that involve soil structure formation, decomposition of organic matter, recycling of important elements, solubilisation of mineral nutrients, producing plant growth regulators, degrading organic

pollutants, crucial for soil fertility, bio control of soil and seed borne plant pathogens and in promoting changes in vegetation (Sivasakthi *et al.,* 2014).

Considerable studies has demonstrated their potential utility, the successful application of plant growth promoting rhizobacteria in the field has been limited by a lack of knowledge of ecological factors that determine their survival and action in the plant rhizosphere. To be effective, PGPR must keep a critical population density of active cells. Inoculation with PGPR strains can temporarily improve the population size, but inoculants often have poor survival and struggle with indigenous bacteria for available growth and experimental evidences suggests that the plant growth stimulation is the net result of multiple mechanisms of action that may be activated simultaneously (Martinez *et al.,* 2010b).

Nowadays PGPR are commonly used in developing countries, and inoculants are used on millions of hectares of land (Zehnder *et al.,* 2001). Nevertheless, performance of this biotechnology has been hindered by the lack of consistency and variation in responses that are obtained in field trials from site to site, year to year, or for different crops. Successful establishment of the introduced bacteria depends on proper PGPR selection that must be tailored to the soil and crop combination. Other basic problems that are related to inoculums production, delivery and storage have mostly precluded the use of non-spore forming bacteria as soil inoculants. Lastly, there has been considerable confusion over the precise effects of PGPR, which confounds scientific studies aimed at quantifying their contribution to plant growth. This is largely due to poor understanding of the interactions between PGPR and their plant hosts and the resident micro flora, as well as a scarcity of information on how environmental factors influence process that contribute to plant growth promotion (Martinez *et al.*,2010b).

The use of *Pseudomonas fluorescence* as PGPR and /or biological control agent requires the precise understanding of the interaction between plant-bacteria, among bacteria-micro biota and how biotic and a biotic factors influence in this relationship. In a few years, current technologies, such as immunofluorescence microscopy and reporter genes, have enhanced the study of *pseudomonas* inoculants in soil and have markedly enhanced the knowledge about their behaviors in this environment (Gotz *et al.,* 2006). It is important to better understand the plant response to the occurrence of these introduced bacteria (e.g. Presence in the high concentration, as in the inoculants). An important concern is the characterization of the rhizosphere populations. Comprehensions of the dynamics of the microbial populations could

shed light in the process to select successful strains promoting plant growth and/ or suppressing diseases (Botelho and Mendonça-hagler, 2006).

Another essential concern is the impact of microorganism massive introduction can cause to the soil ecosystem. It is essential to evaluate the possible impact on the native communities and the resulting effects. Much research had analyzed the impact and the survival and ability to compete of these microorganisms. (Botelho *et al.,* 1998), Observed no variation in the size, structure and function of bacteria communities analyzed when a strain of *Pseudomonas fluorescence* was introduced by seeds of maize. Strains of *Pseudomonas fluorescence* and its genetically modified organisms were able to survival and/or colonize bulk soil and rhizospheric of maize (Guimaraes *et al.*,1997).

2.4. Commercial use of PGPR in Agriculture

The development of biological products based on beneficial microorganisms can extend the range of options for maintaining the health and yield of crops. The commercialization of PGPR strains depend on the linkages between the scientific organizations and industries. Commercial success of PGPR strains requires economical and viable market demand, safety and stability, consistent and broad spectrum action, longer shelf life, low capital costs and easy availability of career materials (Gupta *et al.*, 2015).

Commercial development of agricultural product must follow several criteria: large application on major crops, quality control, inoculums formulation and chemical effects. Product safety, production costs, effectiveness against target organism and value of crops to be treated must all be considered in the development of biological control and PGPR (Botelho and Mendonça-hagler, 2006). Many factors that may affect rhizosphere microbial communities and it is likely different soils, varieties, climatic conditions, etc. will have effect on PGPR performance. Strategies like, use of mixtures of strains improve rhizosphere colonization (Pierson *et al.,* 1994). In addition to physical, environmental, microbial and variety factors that may affect PGPR, other characteristics must be examined before large-scale commercial production will be practical. Strains of *Pseudomonas* species can mutate in culture and generally lose viability when stored for a period of several weeks (Haas *et al.,* 2005).

There is very limited knowledge of the use of rhizobacteria in agriculture in Ethiopia. Interactions among PGPR and plants are still not well understood, especially in field application and different environments in Ethiopia. The increase in the price of chemical fertilizer, the lack of fertilizer industries in developing countries and the growing environmental concerns have concentrated the attention of scientists worldwide on bio fertilizers / PGPR. There is no inoculants industry in Ethiopia but there has been an enormous increase of interest in research in recent years in this area. This increase in interest and the limited usage of chemical fertilizer in the country gives the development of bio fertilizers/ PGPR great opportunities. In order to develop *Pseudomonas fluorescence* as bio control agents for commercial use, a number of organisms must be isolated from the rhizosphere of cereals and assessed for their effectiveness against an important plant pathogen and growth promoting agents (Idris *et al.,* 2009).

2.5. Statement of the problem

Most of the soils that farmers in Ethiopia use for crops agricultural lack sufficient amounts of nutrients such as nitrogen, phosphorus and iron to support optimal plant growth. Since the "Green revolution" in the 1950s, farmers around the world have been using chemical products to obtain high yields from crops. The utilization of chemical fertilizers along with fungicides, herbicides, and insecticides has in recent times been described as causing long term harmful effects on the environment. However, in Ethiopia and more generally Africa's case most of farmers do not use chemical fertilizer since it is not economically viable. Therefore the farming communities experience low yield from their crop harvest. Currently, in the context of both the environmental impact of chemical and cost of fertilizers, excessive reliance on the chemical fertilizers is not viable strategy in long run. There is a very little information concerning the use of PGPR as bio fertilizers in plant growth promoter and plant health.

The repeated applications of fertilizers lead to the loss of soil fertility, disturbance to microbial diversity and their associated metabolic activities. In order to enhance the soil fertility, the producers use diverse mineral fertilizers, that caused several environmental and health damages. Also, the intensive use of mineral fertilizers without addition of organic substance leads to poor soil organic content, which is more sensitive to rain erosion and wind. To overcome the above problems, environmental advocates recommended the use of beneficial soil microorganisms (Agbodjato *et al.,* 2015). At present farming practices have to be make ground on developing agricultural productivity; but the lessons from green revolution have indicated that we should direct our attention to different agricultural crop enhancing techniques, this is where PGPR and bio control bacteria can play a role (Chianu *et al.,* 2011). Therefore, the present study is undertaken to isolate *Pseudomonas fluorescence* that is compatible with cereals and also investigate the effect of *Pseudomonas fluorescence* on the seed germination and growth of maize, wheat and sorghum seedlings as well.

2.6. Significance of the study

The outcomes of this study will be significant in contributing strategies to utilizing plant-associated bacteria in order to develop productivity of farming and sustainability in Ethiopia. This will contribute to increasing agricultural productivity in Ethiopia. This is done by providing practical and affordable scientific solutions to local farmers. Studies in microbial diversity are important in order to be aware of the role of microorganisms in the ecology of soil and other ecosystems. Currently, microorganisms play a significant role in agricultural system, particularly the group of bacteria plant growth promoting rhizobacteria.

Commercialization of biological inoculants increases the mobilization of key nutrients especially P, and enhances their availability to crop plants.

The application of PGPR in cereal crops as inoculants is being very attractive since it would substantially reduce the use of chemical fertilizers and pesticides. With the use of PGPR gaining acceptance, numerous bacterial species have been isolated and their capacity to promote plant growth and development has been investigated. In the search for efficient PGPR strains with multiple attributes, various genera of bacteria show promising results.

3. OBJECTIVES

3.1. General objective

The general objective of the study was to assess the role of *Pseudomonas fluorescence* on growth promoting of maize, wheat and sorghum.

3.2. Specific objectives

- ✓ To isolate and characterize *Pseudomonas fluorescence* from rhizosphere of maize, wheat and sorghum.
- ✓ To evaluate the antagonistic activities of the isolated bacteria against *Fusarium oxysporum.*
- ✓ To evaluate the effect of selected *Pseudomonas fluorescence* strains on seed germination and growth parameters of maize, wheat and sorghum.

4. MATERIALS AND METHODS

4.1. Study area

Rhizospheric soil samples were collected in polyethylene bags from fields of growing of maize, wheat and sorghum from two kebales of Debark district, North Gondar, Amhara Regional State. Because of the continuous cultivation, use of synthetic products in highlands like Migara and Debir, there is a problem of land degradation which cause decrease in nutrients. Migara found at altitude of 2772m above sea level, Debir at altitude of 2840m above sea level. Debark is located in 13°9'22"N, 37°53'53.02"E (Assefa *et al.*, 2010).

4.2. Study design and period

The study design were complete random design (CRD) based experimental. Each treatment had three replications and each experiment was triplicate. Data from pot experiments were used to evaluate the effect of bacterial suspension on maize; sorghum and wheat seedlings and are quantitative in nature. Study has been begun on January, 2016 and completed on June 2017.

4.3. Collection of sample and sampling techniques

Maize, wheat and sorghum were selected randomly in the field and the sampling methods used in the farming lands are convenience sampling with plant health scale that was based on the physical appearance of the plants. The rhizospheric soils were carefully taken in plastic bags and labeled well. The rhizospheric soils samples were used for isolation and characterization of *Pseudomonas fluorescence*. This was done in Microbiology Laboratory, Department of Biology, and College of Natural and Computational science, University of Gondar.

4.4. Sample size

Samples were collected from the upper 30-50cm of the soil profile by digging up a whole plants and removing the non-rhizospheric soil. 900g of rhizospheric soil samples were taken from farming lands. The soil sample taken from that subsistence farming lands were bulked to make compost soil samples.

4.5. Isolation of *Pseudomonas fluorescence* from the soil sample

Pseudomonas fluorescence from the soil samples were isolated through standard serial dilution and pure culture method (El-meleigi, 1989). In laboratory, 1 g of soil sample was put in test tube containing 9ml distilled water and shook with a shaker. Different concentrations

were prepared from each tube, in serial form, and 0.1ml of each concentration was poured in King B medium Petri plates were incubated in 25-28^{o}C for 48h. After colonies formation, petri plates with the best concentration were selected and fluorescent colonies were determined using 366nm UV ray. The other colonies were examined under 10 × magnification and representatives from each morphology distinct group were transferred into Kings B tubes. Bacterial cultures were stored at 4^{o}C and transferred monthly to new media. Similar isolation was conducted from each site at 4-week intervals during the growing season (Alipour and Sobhanipour, 2012).

4.6. Characterization of *Pseudomonas fluorescence*

Microorganisms characterized and identified using colony morphology, pigmentation, and biochemical characteristics. The bacterial isolated were subjected to Gram staining and specific biochemical test (Suman *et al.,* 2016).

4.6.1. Gram Staining

Gram staining reactions were carried out in order to differentiate the bacteria as Gram positive and Gram negatives.

4.6.2. Biochemical test

Biochemical tests were done for the conformation of microorganisms. The tests which are conducted in this experiment are indole test, catalase test, citrate utilization test, methyl red test, amylase test, triple sugar iron / H_2S test, urease test, gelatin hydrolysis, starch hydrolysis and motility test (Shurti *et al.,* 2013).

4.7. *In vitro* fungal inhibition assay

The bacteria isolated from the rhizosphare of the three crops were tested for their efficacy as bio control agent against *Fusarium oxysporium.* Actively growing *Fusarium oxysporium* placed in the center of Petri plates containing Potato Dextrose Agar (PDA). A loop full actively growing *Pseudomonas fluorescence* was spotted in opposite to the *Fusarium oxysporium*. Plates inoculated with *Fusarium oxysporium* and without *Pseudomonas fluorescence* were used as control. After an incubation of ten days at a temperature of 28^{o}C, the diameters of inhibition zone were recorded and inhibition percentages were calculated.

Inhibition zone was calculated by the following formula.

Inhibition percentage% = (A1−A2/ A1) ×100

Where, A1 = radial growth of pathogenic mycelia without bacteria isolates (mm)

A2 = radial growth of pathogenic mycelia with bacteria isolates (mm) (Fouzia *et al.,* 2015).

4.8. In vitro seeds germination assays

Germination test were carried out by using paper towel. Seeds were surface sterilized by soaking in sterile distilled water for 10 min followed by a 1 min immersion in 95% ethanol. The seeds were then immediately rinsed with sterile distilled water. Twelve seeds of maize, 12 seeds of sorghum and 12 seeds of wheat inoculated with *Pseudomonas fluorescence* were arranged in equidistance manner in sterile Petri plates and incubated in growth chamber at 28^0C. No treated seeds with any isolate were designated as control. After seven days the numbers of germinated seeds were counted and percent of germinations were calculated. Percent of germination = (Number of seed germinated/ total number of seed planted) × 100. There is also control group without inoculating with the bacteria (Kochoni *et al.,* 2013).

4.9. Pot Experiment

Roles of *Pseudomonas fluorescence* in maize, wheat and sorghum were evaluated in pots. The experiment include the following treatments: Non-infested soil (control), soil treated with *Pseudomonas fluorescence* isolates, pots were kept under pot experiment conditions till the end of the experiment (Abd-El-Khair *et al.,* 2010). Three isolates of *Pseudomonas fluorescence* from different soil samples were used. This means from maize, wheat and sorghum. The explanation for the effectiveness of this particular combination is that these isolates do not compete for space and together colonize the root surface more effectively than single isolates (Hol *et al.,* 2013).

Pseudomonas fluorescence (P.f1) which was isolated from rhizosphere of maize,

Pseudomonas fluorescence (P.f2) which was isolated from rhizosphere of wheat, and

Pseudomonas fluorescence (P.f3) which was isolated from rhizosphere of sorghum, and the seeds were inoculated by mixing the three *Pseudomonas fluorescence* (P.f1, P.f2 and P.f3 interchangeably. Therefore, there are seven treatments for each of maize, wheat and sorghum seeds.

Treatment 1: Seeds + (P.f1, P.f2 and P.f3)

Treatment 2: Seeds + (P.f1 and P.f2)

Treatment 3: Seeds + (P.f1 and P.f3)

Treatment 4: Seeds + (P.f2 and P.f3)

Treatment 5: Seeds + P.f1

Treatment 6: Seeds + P.f2

Treatment 7: Seeds + P.f3 and also there are control group for each treatments.

Growth promoting ability of the bacterial isolates was determined based on the data collected on root lengths, shoot height, shoot fresh weight, root dry weight, shoot dry weight and overall growth.

4.9.1. Inoculums preparation

Bacteria used for seed inoculations were grown in KB medium at 27^{o}C for two days or until there was good growth. For growth chamber studies, bacteria were cultured on nutrient broth for 48 h at 25 to 27^{o}C. Mixtures of bacterial strains (P.f1 +P.f2+P.f3) were prepared by mixing 1:1:1 volume suspensions. Single well isolated colonies were picked up with sterile loop and inoculated in 6ml nutrient broth and incubated at 27^{o}C or seed were coated with bacteria. But before coating seed with bacteria or preparation of suspension, seeds were surface sterilized using the following procedure. Seeds were soaked in sterile distilled water for 10 min followed by a 1 min immersion in 95% ethanol. The seeds were then immediately rinsed with sterile distilled water (Idris *et al.,* 2009).

4.10. Plant growth promotion in pots

The effects of isolated bacteria on plants growth were studied in pot experiments. The inoculation treatments were setup in a randomized design. The day before sowing, pots were filled with 350g soil, which was collected from 0-15/30cm depth from farmer's field. The soil was moistened with water and maintained at 60% of its moisture holding capacity (MHC). Three seeds of maize, three seeds of wheat and three seeds of sorghum were sown per pots. The controls were considered as un-inoculated plants. The bacterial inoculums (30ml of the 10^{6}-10^{8} cfu/ml suspension) was applied as a soil drench once a week for two weeks. Plants were grown in pots for 4 weeks under pot experiment conditions with a temperature of 32-34^{o} C during the day and 18-22^{o} C at night. The seeds were watered regularly until the emergence of the first shoot. Seven days later, 30ml of the bacterial inoculums was applied to the pots as a soil drench and a second application was made 1 week later. The pots were watered twice daily. Four weeks after germination, the shoot length, shoot fresh weight, shoot dry weight, root length and root dry weight and overgrowth were measured, separated, and dried at 105^{o}C , before determining their respective dry weights (Egamberdiyeva, 2005).

4.11. Data collected

Morphological, cultural and biochemical characteristics of the bacteria isolated from the soil sample were recorded. The bacterial inhibitions against *Fusarium oxysporium* were observed and clear zone of inhibition were checked. And also the effects of bacterial inoculation on the germination of the seeds were observed on Petri dish. In the pot experiment the effect of bacteria on shoot height, shoot fresh weight, root lengths, root dry weight, shoot dry weight were observed and the data collected by measuring using cm unit.

4.12. Data analysis

The data recorded were subjected to analysis using the appropriate soft ware. ANOVA was conducted using IBM SPSS version 20. To determine the significant difference between the means followed by Duncan's multiple range tests ($p \leq 0.05$). Statistical significant difference were determined at $p <$ or equal to 0.05.

5. RESULTS

5.1. Isolation and characterizations of *Pseudomonas fluorescence* strains.

Pseudomonas strains showed *fluorescent* colour on Kings B medium under U.V light. From nine isolates of *Pseudomonas fluorescence* three isolations were effectively selected from the rhizosphere of the three cereal crops. They were designated as P.f1 (*Pseudomonas fluorescence* 1*)*, P.f2 (*Pseudomonas fluorescence 2)*, and P.f3 (*Pseudomonas fluorescence 3)* isolated from maize, wheat and sorghum rhizosphere respectively. *Pseudomonas* species isolated were identified by morphological and physiological characteristics based on Bergeys' Manual of Systematic Bacteriology. All strains were yellow green pigment and under microscope they were gram negative and morphologically rod-shaped. They were separated into P.f1, P.f2 and P.f3 based on colony size and cereal crops. Results of biochemical characterization of *Pseudomonas fluorescence* species indicates that all the three isolates of *Pseudomonas fluorescence* strain were negative for urease test, indole test, methyl red test, gelatin hydrolysis and starch hydrolysis and positive for catalase, citrate utilization test and triple sugar iron tests.

5.2. Antifungal activity

All *Pseudomonas fluorescence* strains tested inhibited the growth of the fungus: the inhibition rate varied according to the bacterial strain and the fungus. Mycelia growth of *Fusarium oxysporum* was strongly reduced in the presence of antagonistic *Pseudomonas fluorescence* strains, compared with the uninoculated control as shown in (Table 1). For treatment one (T1) the inhibition percentage was calculated as: A1 = 90mm, A2 = 61.34 mm.

Inhibition percentage % = (90 – 61.34) / 90× 100

= 31.84 %

All the isolates found to inhibit growth of *Fusarium oxysporium.* As shown in table there is highly significant variation at p<.001 betweenT1, and other treatments. The highest zone of inhibition agianst *Fusarium oxysporium* was shown by treatment one (T1) (28.67mm) followed by treatment two and three (T2 and T3). Treatment five, six and seven (T5,T6 and T7) shows the lowest antagonistic effect against *Fusarium oxysporium.* The results expressed as efficacy test, were calculated using the following formula.

Efficacy test = [(Mycelia growth of the control – Mycelia growth of treatment) / Mycelia growth control] × 100 %.

Table 1 Effect of *Pseudomonas fluorescence* on fungal inhibition

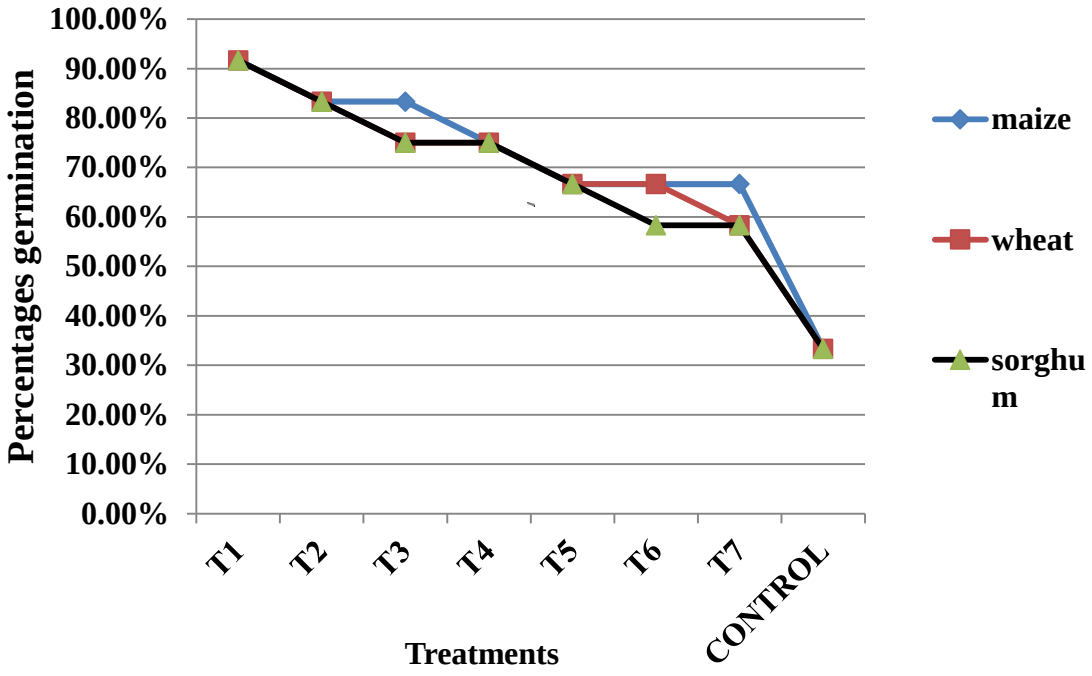
Treatments	Mycelia growth of *Fusarium oxysporium* (mm)	Mean of *Fusarium Oxysporium* inhibited (mm)	Percent inhibition over control	Efficacy test
T1	61.34	28.67[c]	31.84	31.84%
T2	76.00	14.00[b]	15.55	15.55%
T3	75.67	14.33[b]	15.92	15.92%
T4	76.34	13.67[b]	15.17	15.17%
T5	78.67	11.33[a]	12.58	12.58%
T6	78.00	12.00[a]	13.33	13.33%
T7	79.34	10.67[a]	11.84	11.84%
Control	90.00	_	_	
	LSD (5%) =1.576	Least significant differences of means (5% level)		
	CV (%) = 6.0	Coefficients of variation		

Values represent average of three replications. Distance between the edges of fungal mycelia and the bacterial streak. Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Tests at *P*=0.05. Note: T1= (P.f1+P.f2+P.f3), T2= (P.f1+P.f2), T3= (P.f1+P.f3), T4= (P.f2+P.f3), T5= P.f1, T6= P.f2 and T7= P.f3.

5.3. Seeds germination assay

Pseudomonas fluorescence isolates remarkably affected the germination of maize, wheat and sorghum seeds. As shown in figure 1 percent of germination test was calculated as: Percent of germination = (Number of seed germinated / total number of seed planted) × 100. Treatment one (T1) show the highest germination percentage for all the crops (91.66%).

That means, from twelve seed planted only eleven seed germinated from each crops. Therefore, Percent of germination (T1) = (11/ 12) ×100 = 91.66%. The lowest germination percentages indicated by the control group (33.33%) for each crop. This means from total of twelve seed planted only 4 seed germinated from each crops.



Note: T1= (P.f1+P.f2+P.f3), T2= (P.f1+P.f2), T3= (P.f1+P.f3), T4= (P.f2+P.f3), T5= P.f1, T6= P.f2 and T7= P.f3.

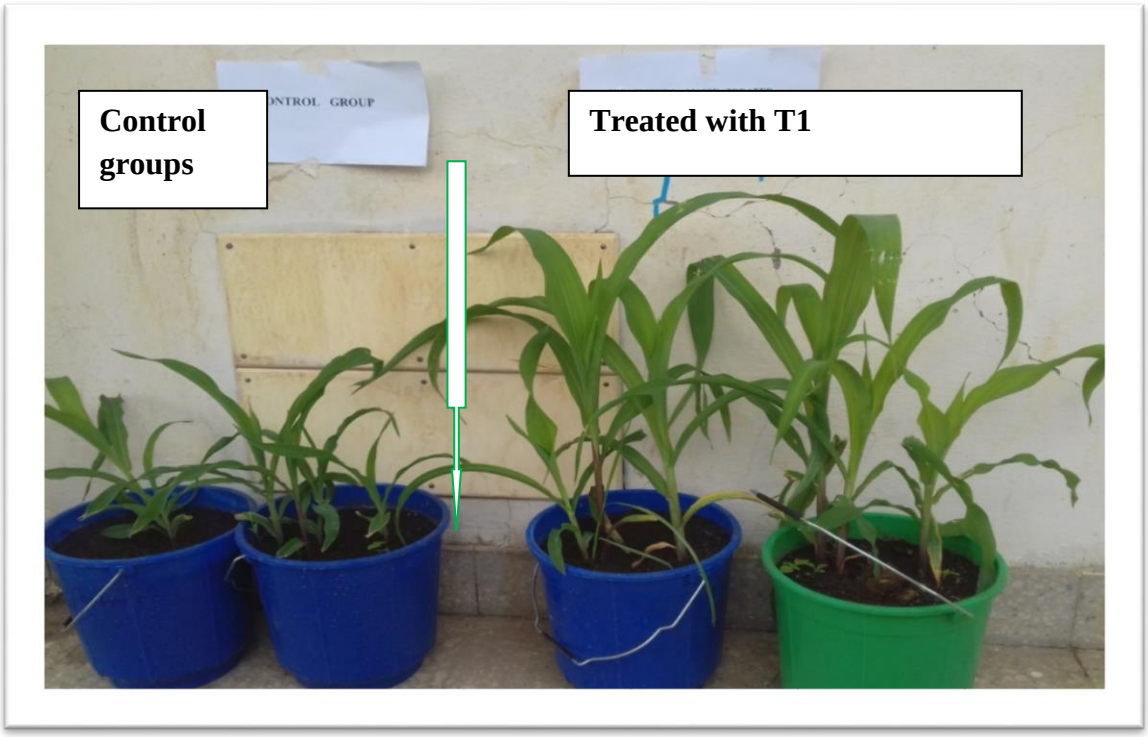
Figure 1 Effect of *Pseudomonas fluorescence* on seed germination (%).

5.4. Pot experiments

The effects of *Pseudomonas fluorescence* (PGPR strain) on shoot height, shoot fresh weight, shoot dry weight, root length and root dry weight and overall growth were evaluated as the following. Periodical data on growth parameters were recorded at 12, 17, 22, 27 and 32 days interval.

5.4.1. Shoot height

The *Pseudomonas fluorescence* isolates significantly affected the height of maize seedlings at $p < .001$ (Table 2), wheat seedlings at $p < .001$ (Table 3) and sorghum seedlings at $p < .001$ (Table 4) over the control groups. The results of shoot heights of maize, wheat and sorghum were collected within 5 days intervals. It started from the growth of the seeds after 12 days. Those are at days 12, 17, 22, 27 and 32. Plant growth promotion of maize (Figure .2.), wheat (Figures.3) and sorghum (Figure. 4) with *Pseudomonas fluorescence* strains after 32 days over control groups.



Note: T1= (P.f1+P.f2+P.f3)

Figure 2 Plant growth promotion of maize in pots (32 days after sowing)

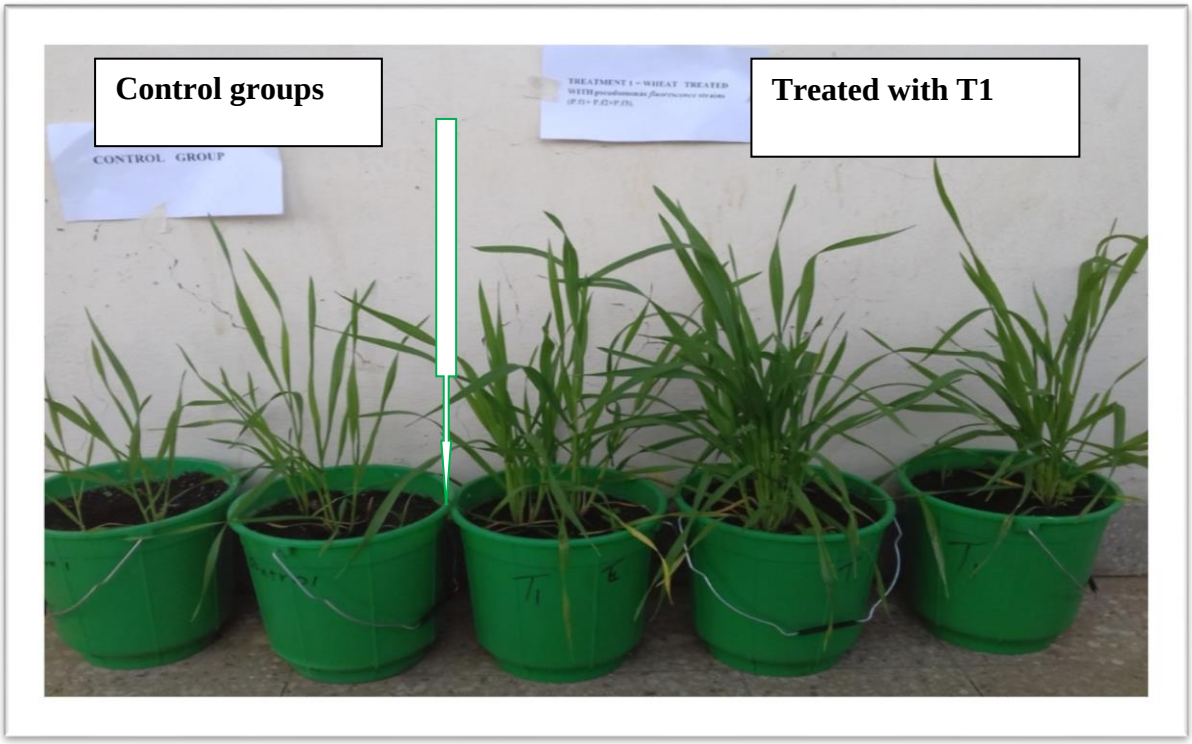
Results indicate that shoot height of maize seedling increased in *Pseudomonas fluorescence* strain treated plants over uninoculated control. The highest shoot height (24.33cm) was recorded in treatment one (T1) which was statistically similar to treatment two (T2) (23.67cm) after 32 days. The lowest shoot height (14.80cm) was recorded in control groups after 32 days. At the day 12 all treatments are statistically similar (Table 2).

Table 2 Effect of *Pseudomonas fluorescence* on shoot height (cm) of maize within 5 days interval

Treatments	Days				
	D12	D17	D22	D27	D32
T1	5.00^{a}	15.67^{f}	19.33^{ij}	21.33^{mn}	24.33^{r}
T2	4.67^{a}	15.17^{ef}	18.67^{hi}	20.67^{lm}	23.67^{qr}
T3	4.67^{a}	15.17^{ef}	18.33^{gh}	20.33^{kl}	23.33^{pq}
T4	4.67^{a}	14.83^{ef}	18.00^{gh}	20.00^{jkl}	23.00^{pq}
T5	4.17^{a}	14.67^{e}	17.67^{g}	19.67^{jk}	22.67^{op}
T6	4.17^{a}	14.50^{e}	17.67^{g}	19.67^{jk}	22.67^{op}
T7	4.33^{a}	14.50^{e}	17.67^{g}	19.67^{jk}	22.00^{no}
Control(cm)	4.17^{a}	7.83^{b}	9.67^{c}	10.93^{d}	14.80^{ef}

∗LSD (5%) =0.8505 ∗ Least significant differences of means (5% level)
∗∗ CV (%) = 3.4 ∗∗ Coefficients of variation

Note: a-r Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.



Note: T1= (P.f1+ P.f2+P.f3)

Figure 3 Plant growth promotion of wheat in pots (32 days after sowing)

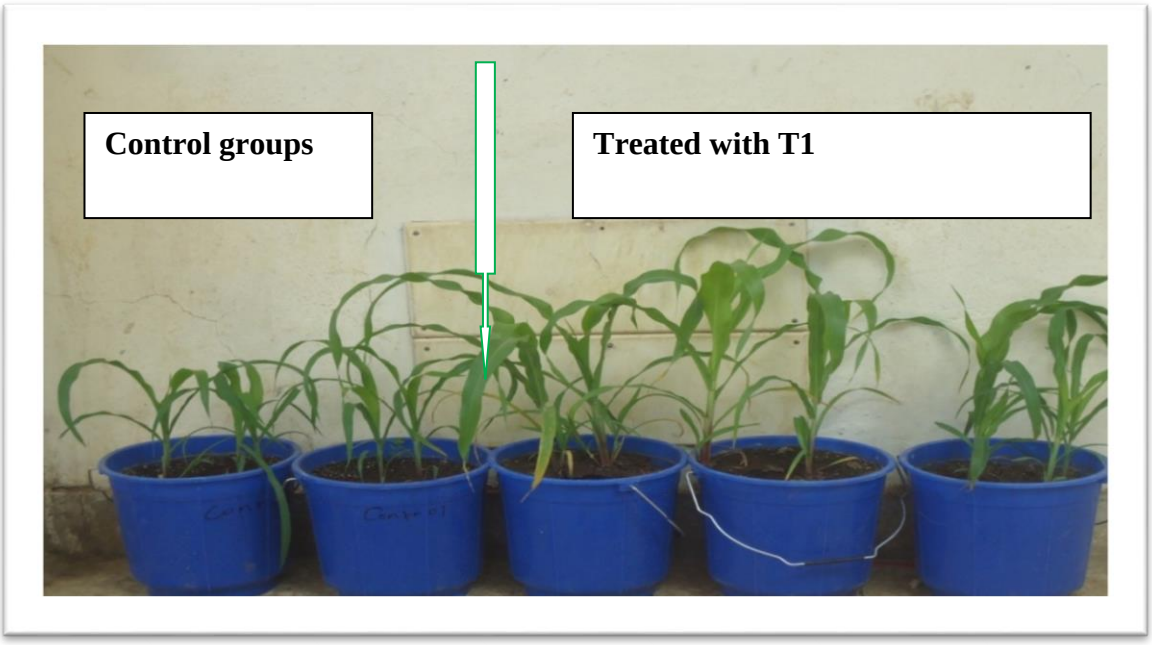
Results indicate that shoot height of wheat seedlings increased in *Pseudomonas fluorescence* strain treated plants over uninoculated control. The highest shoot height (17.67cm) was recorded in treatment one (T1) which was statistically similar to treatment two (T2) (17.33cm), treatment three (T3) (17.00cm) and treatment four (T4) (17.33cm) after 32 days. The lowest shoot height (9.17cm) was recorded in control groups after 32 days (Table 3).

Table 3 Effect of *Pseudomonas fluorescence* on shoot height (cm) of wheat within 5 days interval

	Days				
Treatments	D12	D17	D22	D27	D32
T1	7.67^{cd}	12.67^{hij}	13.67^{klm}	14.67^{nop}	17.67^{r}
T2	7.67^{cd}	12.33^{hi}	13.33^{jkl}	14.33^{mno}	17.33^{r}
T3	7.00^{bc}	12.40^{hij}	13.00^{ijk}	14.00^{lmn}	17.00^{r}
T4	7.17^{bc}	12.67^{hij}	13.33^{jkl}	14.33^{mno}	17.33^{r}
T5	6.83^{bc}	11.33^{fg}	12.33^{hi}	13.33^{jkl}	15.67^{q}
T6	6.37^{b}	10.67^{f}	12.00^{gh}	13.00^{ijk}	15.00^{opq}
T7	6.27^{b}	11.33^{fg}	12.33^{hi}	13.33^{jkl}	15.33^{pq}
Control(cm)	3.50^{a}	6.33^{b}	7.33^{c}	8.47^{de}	9.17^{e}

∗ LSD (5%) = 0.828 ∗ Least significant differences of means (5% level)
∗∗CV%= 4.3 ∗∗ Coefficients of variation

Note: a-r Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.



Note: T1= (P.f1+ P.f2 +P.f3)

Figure 4 Plant growth promotion of sorghum in pots (32 days after sowing)

Results indicate that shoot height of sorghum seedling increased in *Pseudomonas fluorescence* strain treated plants over uninoculated control. The highest shoot height (15.00cm) was recorded in treatment one (T1) which was statistically similar to treatment two (T2) (15.00cm) after 32 days. The lowest shoot height (7.20cm) was recorded in control groups after 32 days (Table 4).

Table 4 Effect of *Pseudomonas fluorescence* on shoot height (cm) of sorghum within 5 days interval

	Days				
Treatments	D12	D17	D22	D27	D32
T1	4.27^{c}	7.33^{ef}	9.33^{gh}	11.17^{i}	15.00^{m}
T2	3.83^{bc}	7.00^{de}	9.00^{g}	10.67^{i}	15.00^{km}
T3	3.90^{c}	7.00^{de}	8.67^{g}	10.53^{i}	14.00^{k}
T4	3.93^{c}	7.00^{de}	8.67^{g}	10.67^{i}	14.00^{kl}
T5	3.77^{bc}	6.77^{de}	8.33^{g}	10.33^{i}	13.00^{j}
T6	3.67^{bc}	6.20^{d}	8.33^{g}	10.33^{i}	12.33^{j}
T7	3.77^{bc}	6.57^{de}	8.33^{fg}	10.33^{hi}	12.67^{j}
Control (cm)	2.07^{a}	2.80^{ab}	4.20^{c}	6.17^{d}	7.20^{de}

∗ LSD (%) = 0.9407 ∗ Least significant differences of means (5% level)
∗∗CV% = 7.1 ∗∗ Coefficients of variation

Note: a-m Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.

5.4.2. Shoot fresh weight

A significant increase in shoot fresh weight of maize at p < .001, wheat at p < 0.001 and sorghum at p < 0.002 seedlings was observed in response to *Pseudomonas fluorescence* isolates over control groups. The significant increase in shoot fresh weight was observed in response to treatment 1 (T1). It increased shoot fresh weight by 12.83g, 23.00g and 12.00g in maize, wheat and sorghum respectively over the uninoculated control of maize (7.37g), wheat (8.03g) and sorghum (6.77g) respectively (Table .5).

Table 5 Effect of *Pseudomonas fluorescence* on shoot fresh weight

	Mean weight in grams		
Treatments	Maize	Wheat	Sorghum
T1	12.83^{d}	23.00^{d}	12.00^{c}
T2	11.70^{cd}	19.67^{c}	10.67^{bc}
T3	11.37^{bc}	20.33^{c}	9.67^{b}
T4	11.17^{bc}	19.33^{c}	10.67^{bc}
T5	10.47^{bc}	15.00^{b}	9.97^{b}
T6	10.43^{bc}	14.33^{b}	9.50^{b}
T7	10.23^{b}	14.00^{b}	9.60^{b}
Control(g)	7.37^{a}	8.03^{a}	6.77^{a}
LSD (5%)∗	1.415	1.769	1.841
CV (%)∗∗	7.6	6.1	10.8

Note:∗ Least significant differences of means (5% level) ∗∗ Coefficients of variation

Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.

5.4.3. Shoot dry weight

A significant increase in shoot dry matter of maize, wheat and sorghum seedlings at p < 0.001 was observed in response to *Pseudomonas fluorescence* isolates over control groups. Significant increase in shoot dry matter was observed in treatment 1 (T1). That was 5.47g, 5.57g and 2.63g in maize, wheat and sorghum respectively over the uninoculated control of maize (1.6g), wheat (1.7g) and sorghum (1.3g) respectively (Table .6.).

Table 6 Effect of *Pseudomonas fluorescence* on shoot dry weight

Treatments	Mean weight in grams		
	Maize	Wheat	Sorghum
T1	5.47^{f}	5.57^{g}	2.63^{f}
T2	3.57^{e}	4.17^{f}	2.40^{e}
T3	3.17^{d}	4.27^{f}	2.33^{de}
T4	3.00^{c}	3.70^{e}	2.40^{e}
T5	2.90^{c}	3.50^{d}	2.20^{cd}
T6	2.60^{b}	3.20^{c}	2.00^{b}
T7	2.50^{b}	3.00^{b}	2.10^{bc}
Control(g)	1.60^{a}	1.70^{a}	1.30^{a}
LSD (5%)∗	0.149	0.149	0.180
CV (%)∗∗	2.8	2.4	4.8

Note:∗ Least significant differences of means (5% level) ∗∗ Coefficients of variation
Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.

5.4.4. Root length

A significant variation in root length of maize, wheat and sorghum at $p < 0.001$ was observed in response to different *Pseudomonas fluorescence* isolates over control. The highest root length was observed in treatment 1(T1). That was 58.67cm, 49.00cm and 55.00cm in maize, wheat and sorghum respectively over uninoculated control of maize (33.00cm), wheat (30.00cm) and sorghum (28.00cm) respectively (Table.7.).

Table 7 Effect of *Pseudomonas fluorescence* on root length

Treatments	Mean length of root (cm)		
	Maize	Wheat	Sorghum
T1	58.67^{e}	49.00^{d}	55.00^{f}
T2	56.00^{d}	46.00^{bc}	53.33^{ef}
T3	55.00^{bcd}	46.00^{bc}	52.67^{de}
T4	55.33^{cd}	46.67^{c}	52.33^{cde}
T5	53.67^{bc}	44.33^{b}	51.00^{bcd}
T6	53.67^{bc}	44.33^{b}	50.67^{bc}
T7	53.33^{b}	44.67^{b}	49.67^{b}
Control(cm)	33.00^{a}	30.00^{a}	28.00^{a}
LSD (5%)∗	1.903	1.967	1.903
CV (%)∗∗	2.1	2.6	2.2

Note:∗ Least significant differences of means (5% level) ∗∗ Coefficients of variation

Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.

5.4.5 Root dry weight

The *Pseudomonas fluorescence* isolates significantly increased the root dry weight of maize, wheat and sorghum at $p < 0.001$ over control groups. It was increased by 1.5g, 1.7g and 1.3g in maize, wheat and sorghum respectively over the uninoculated control of maize (0.43g), wheat (0.57g) and sorghum (0.47g) (Table .8.).

Table 8 Effect of *Pseudomonas fluorescence* on root dry weight

	Mean weight in grams		
Treatments	Maize	Wheat	Sorghum
T1	1.50^{e}	1.70^{f}	1.30^{e}
T2	1.03^{d}	1.50^{e}	1.07^{d}
T3	1.00^{d}	1.43^{de}	0.97^{cd}
T4	0.90^{cd}	1.37^{d}	0.80bc
T5	0.80^{bc}	1.03^{c}	0.77^{b}
T6	0.77^{bc}	0.90^{b}	0.77^{b}
T7	0.73^{b}	0.87^{b}	0.73^{b}
Control(g)	0.43^{a}	0.57^{a}	0.47^{a}
LSD (5%)∗	0.165	0.132	0.173
CV (%)∗∗	10.7	6.5	11.7

Note: ∗ Least significant differences of means (5% level)

∗∗ Coefficients of variation

Means in a column followed by same superscript letters are not significantly different according to Duncan's Multiple Range Test at *P*=0.05.

6. DISCUSSION

Pseudomonas fluorescence strains, normally present in rhizospheric soils of cereal crops are effective colonizers of the rhizosphere of many crop plants possessing potential to increase and inhibit growth of number of pathogenic fungi. Results revealed that all the three isolates of *Pseudomonas fluorescence* were negative for urease test, indole test, methyl red test, gelatin hydrolysis and starch hydrolysis and positive for catalase, citrate utilization test and triple sugar iron tests. Similar results were obtained with Shurti *et al.* (2013),who isolated *Pseudomonas fluorescence* from the rhizospheres of rice from various parts of Bhojia institute of life sciences, Budh (Baddi), Himachal Pradesh garden, later on confirmed the fluorescent colonies by viewing under UV-light.

The isolates induced inhibition zones ranging from 10 mm-29mm towards the *Fusarium oxysporium*. There is highly significant variation betweenT1, and other treatments. Higher percentage of mycelia growth inhibition was observed with treatment one (T1) (28.67mm). This means fungal treated with the mixtures of *Pseudomonas fluorescence* strains isolates of (P.f1+ P.f2+P.f3). *Pseudomonas fluorescence* isolates individually, P.f1, P.f2 and P.f3 represent the lowest antagonistic action 11.33mm, 12.00mm and 10.67mm respectively as shown in Table.1. A significant variation in fungal inhibition was observed in response to different *Pseudomonas fluorescence* isolates. Mixtures of *Pseudomonas fluorescence* were significantly more suppressive of take –all than either used alone. Similar antifungal activity exhibited by *Pseudomonas fluorescence* was reported by Showkat *et al.* (2012) and Fouzia *et al.* (2015).

The germination test in controlled condition shows that all *Pseudomonas fluorescence* strains significantly increased germination of crops. Our data revealed that *Pseudomonas fluorescence* treatments promote maize, wheat and sorghum seed germinations. The highest germination percentage 91.66% was observed with the seeds inoculated with the combination of the three isolations of the bacteria in treatment one (T1). The rest treatments also showed the better performances to increase the seed germination. In contrary the lowest germination percentage was observed in the control group (33.33%) as indicated in the figure 1. The present investigation confirms the earlier studies. It revealed that under *in vitro* conditions, seed treatment with PGPR strains improve seed germination as reported by Kochoni *et al.* (2013). The role of PGPR in increasing the growth and yield of various crops such as wheat

(Khalid *et al.,* 2004), maize (Silva *et al* ., 2016) and many others has been reported in the past.

It was observed that in pot experiment inoculation with *Pseudomonas fluorescence* strains significantly promoted growth of seedling maize, wheat and sorghum. Significant variation in growth promotion was observed in response to different *Pseudomonas fluorescence* isolates. According to the grouping of isolates using the Duncan's multiple range tests, all treatments resulted in an increase in shoot height, shoot fresh weight, and shoot dry weight, root length, root dry weight and overall growth. The highest increase of maize shoot height was achieved by treatment one (T1) and two (T2) which increased shoot height by 24.33cm and 23.67cm respectively over the uninoculated control (14.80cm) after 32 days. Wheat shoot height increased by 17.67cm and 17.33 treated with treatment one (T1) and two (T2) respectively over uninoculated control (9.17cm). Also the highest increase of sorghum shoot height was achieved by treatment one (T1) and two (T2) which are statistically similar, and increased shoot height by 15.00cm over the uninoculated control (7.20cm) after 32 days (Table 2, 3 and 4). The results obtained in the current study concur with several other studies which elucidated the growth promoting activity of isolates of *Pseudomonas fluorescence.* Shurti *et al.* (2013) reported that inoculation with *Pseudomonas fluorescence* increased overall growth of rice.

The significant increase in shoot fresh weight was observed in response to treatment one (T1). This increased shoot fresh weight by 12.83g, 23.00g and 12.00g in maize, wheat and sorghum respectively over the uninoculated control of maize (7.37g), wheat (8.03g) and sorghum (6.77g) (Table .5). Also the significant increase in shoot dry matter was observed in treatment one (T1) which is a combination of P.f1, P.f2 and P.f3. That was 5.47g, 5.57g and 2.63g in maize, wheat and sorghum respectively over the uninoculated control of maize (1.6g), wheat (1.7g) and sorghum (1.3g) (Table .6.). In a similar study, Idris *et al.*(2009) has reported that the rhizobacteria isolated from the rhizosphere of sorghum in two fields in Ethiopia, in the Meeson and Jijiga areas in the Eastern part of the country, have the ability to enhance growth of sorghum in pot experiments.

A significant variation in root length was observed in response to different treatments of *Pseudomonas fluorescence* strains. The highest root length was observed in treatment one (T1). That was 58.67cm, 49.00cm and 55.00cm in maize, wheat and sorghum respectively over uninoculated control of maize (33.00cm), wheat (30.00cm) and sorghum (28.00cm) (Table.7.). Also a significant increase in root dry matter of the crops was observed in

response to different treatments. This was 1.5g, 1.7g and 1.3g in maize, wheat and sorghum respectively over the uninoculated control of maize (0.433g), wheat (0.567g) and sorghum (0.467g) (Table .8.). The growth promoting of these cereal crops by *Pseudomonas fluorescence* also reported by El-meleigi, (1989).

As the results of the study shows, combined inoculation of *Pseudomonas fluorescence* (P.f1+P.f2+P.f3) isolated from maize, wheat and sorghum increase overall growth than others combinations and isolates. A combination of inoculation of *Pseudomonas fluorescence* isolates is very effective in promoting growth than single isolates. The study by Adesemoye *et al.* (2008) confirmed that inoculation with mixed strains was more efficient than single-strain inoculations The effectiveness of this particular combination is that these isolates do not compete for space and together colonize the root surface more effectively than single isolates (Hol *et al.,* 2013).

7. CONCLUSION

The main conclusions from the current study are that *Pseudomonas fluorescence* isolated from the rhizosphere of maize, wheat and sorghum has the ability to enhance growth of maize, wheat and sorghum under pot experiment conditions. *Pseudomonas fluorescence* inoculants are able to increase plant growth, germination, improve seedling emergence and protect plants from disease. The current study demonstrates that, in general, the mixtures of *Pseudomonas fluorescence* strains provided greater growth promoting than individual strains tested in the pot experiments and also for germination of seeds and antagonistic effects against *Fusarium oxysporium*. Among the evaluated treatments, the combination of P.f1, P.f2 and P.f3 improved shoot height, shoot fresh weight, shoot dry weight, root length and root dry weight of maize, wheat and sorghum. The greater growth response to all inoculants compared to control clearly showed the beneficial role of *Pseudomonas fluorescence*.

Based on these results, it can be concluded that, inoculation of *Pseudomonas fluorescence* strains as bio fertilizer will be useful in increasing maize, wheat and sorghum productivity. Also the present study contributes to the understanding and utilization of *Pseudomonas fluorescence* isolates as bio control agent. Therefore, direct use of *Pseudomonas fluorescence* to promote plant growth and to control plant pests continues to be an area of rapidly expanding research area.

8. RECOMMENDATION

Study on the use of *Pseudomonas fluorescence* inoculants has been conducted under laboratory and pot experiment. Many experiments have demonstrated the growth stimulation of plant crops in the pot experiment, resulting in increased yield parameters and in the control on soil-borne pathogenic organisms. However, the replication of successful results of PGPR applications under field conditions has been limited by the lack of knowledge about their ecology, survival and activity in the plant rhizosphere. However the results presented here should be confirmed through conducting similar studies under field experiments (which involves various soil conditions and agro climates) prior to dissemination to the farmers. Further investigations are also needed to investigate which type of biochemical production is making *Pseudomonas fluorescence* isolates as one of the most suitable candidate in suppressing the pathogenic fungi.

The use of cocktail of *Pseudomonas fluorescence* strains can give potential growth than using one single strain of *Pseudomonas fluorescence*. Formulation, mass culturing and adaptive field trials should be done to make use of them. Molecular characterizations to get information about the *Pseudomonas fluorescence* strains are also needed to make further applications.

9. REFERENCES

Abd-El-Khair, H., Khalifa, R Kh M and Haggag, K., 2010. Effect of *Trichoderma* species on damping off diseases incidence , some plant enzymes activity and nutritional status of bean plants. *Journal of American Science*, **6**(9):486–497.

Adesemoye, A.O., Torbert, H.A. and Kloepper, J.W., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology*, **54**(10):876–886.

Agbodjato, NA., Noumavo, PA., Adjanohoun, A., Dagbenonbakin, G., Atta, M., Rodriguez, AF., Pons, BM and Baba-Moussa, L., 2015. Response of maize (*Zea mays L* .) crop to biofertilization with plant growth promoting rhizobacteria and chitosan un der field conditions. *Journal of Experimental Biology and Agricultural Sciences*, **3**(6):566-574.

Alemu, F., 2014. Isolation of *Pseudomonas fluorescence* species from rhizospheric Soil of health faba bean and assessment of their antagonistic activity and potential of biocontrol under in vivo against botrytis fabae (Chocolate Spot Diseases). *Journal of Health Science*, **2**:75–88.

Alipour, Z.T. and Sobhanipour, A., 2012. The Effect of Thiobacillus and *Pseudomonas fluorescence* Inoculation on Maize Growth and Fe Uptake, **3**(3):1661–1666.

Anitha, G. and Kumudini, B.S., 2014. Isolation and characterisation of *Pseudomonas fluorescence* and their effect on plant growth promotion. *Journal of Environmental Biology*, **35**:627–634.

Ashrafuzzaman, Md., Hossen, Farid Akhtar., Ismail, M Razi., Hoque, A., Islam, M Zahurul ., Shahidullah, SM and Meon, S., 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, **8**(7):1247–1252.

Assefa, B., Glatzel, G. and Buchmann, C., 2010. Ethnomedicinal uses of Hagenia abyssinica (Bruce) J . F . Gmel . among rural communities of Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **6**(1):20.

Barea, Jose-Miguel., Pozo, Maria Jose., Azcon, Rosario and Azcon-Aguilar, C., 2005. Microbial co-operation in the rhizosphere. *Journal of experimental botany*, **56**(417):1761–1778.

Beattie, Gwyn A and Lindow, S.E., 1995. The secret life of foliar bacterial pathogens on leaves. *Annual Review of Phytopathology*, **33**(1):145–172.

Bloemberg, Guido V and Lugtenberg, B.J., 2001. Molecular basis of plant growth promotion

and biocontrol by rhizobacteria. *Current 0pinion in Plant Biology*, **4**(4):343–350.

De Boer, Marjan., van der Sluis, Ientse., van Loon, Leendert C and Bakker, P.A., 1999. Combining fluorescent Pseudomonas spp. strains to enhance suppression of fusarium wilt of radish. *European Journal of Plant Pathology*, **105**(2):201–210.

Botelho, GR., Guimaraes, V., Bonis, M De., Fonseca, MEF., Hagler, AN and Hagler, L., 1998. Ecology of a plant growth-promoting strain of Pseudomonas ¯ uorescens colonizing the maize endorhizosphere in tropical soil. *World Journal of Microbiology and Biotechnology*, **14**(4):499--504.

Botelho, G.R. and Mendonça-hagler, L.C., 2006. Fluoresent pseudomonads associated with the rhizosphere of crops - an overview. *Brazilian Journal of Microbiology*, **37**:401–416.

Chegini, S., Sani, B. and Darvishi, H.H., 2015. The Effect of Pseudomonas Bacteria on Maize Growth and Atrazine Biodegradation in Soil, **7**(1):951–956.

Chianu, Jonas N., Nkonya, Ephraim M., Mairura, FS., Chianu, Justina N and Akinnifesi, F., 2011. Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: a review. *Agronomy for Sustainable Development*, **1**(31):139–154.

Chitra, A., 2015. Multigeneric microbial coaggregates mmc a Novel strategy for the maximization of plant Growth stimulation and induction of Systemic resistance isr against Xanthomonas oryzae pv oryzae In lowland rice.

Cummings, S.P., 2009. The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops ; potential and problems Stephen P. *Environmental Biotechnology*, **5**:43–50.

Djuric, S., Pavic, A., Jarak, M., Pavlovic, S., Starovic, M., Pivic, R and Josic, D., 2011. Selection of indigenous *Pseudomonas fluorescence* isolates from maize rhizospheric soil in Vojvodina as possible PGPR. *Romanian Biotechnological Letters*, **16**(5):6580--6590.

Egamberdiyeva, D., 2005. Characterization of *Pseudomonas* species isolated from the rhizosphere of plants grown in Serozem soil, semi-arid region of Uzbekistan. *The Scientific World Journal*, **5**:501–509.

El-meleigi, M.A., 1989. Effect of soil pseudomonas isolates applied to corn, sorghum and wheat seeds on seedling growth and corn yield. *Journal of plant science*, **69**:101–108.

Fouzia, Arif., Allaoua, Silini., Hafsa, Cherif-Silini and Mostefa, G., 2015. Plant growth promoting and antagonistic traits of indigenous Fluorescent Pseudomonas spp. isolated from wheat rhizosphere and A. Halimus endosphere. *European scientific journal*, **11**(24):129–148.

Ganeshan, G. and Kumar, A.M., 2005. *Pseudomonas fluorescence*, a potential bacterial

antagonist to control plant diseases. *Journal of Plant Interactions*, **1**(3):123–134.

Garcia-Fraile, Paula., Menendez, Esther and Rivas, R., 2015. Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioengineering*, **2**(3):183–205.

Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters*, **251**(1):1–7.

Glick, B.R., Karaturovíc, D.M. and Newell, P.C., 1995. A novel procedure for rapid isolation of plant growth promoting pseudomonads. *Canadian Journal of Microbiology*, **41**(6):533–536.

Gotz, Monika., Gomes, Newton CM., Dratwinski, Albert., Costa, Rodrigo., Berg, Gabriele., Peixoto, R., M. and Hagler, Leda and Smalla, K., 2006. Survival of gfp-tagged antagonistic bacteria in the rhizosphere of tomato plants and their effects on the indigenous bacterial community. *FEMS Microbiology Ecology*, **56**(2):207–218.

Guimaraes, Vanessa F., Cruz, Ivanea V., Hagler, Allen N., Mendoncca-Hagler, Leda C and van Elsas, J.D., 1997. Transport of a genetically modified Pseudomonas fluorescens and its parent strain through undisturbed tropical soil cores. *Applied Soil Ecology*, **7**(1):41–50.

Gupta, Govind., Parihar, Shailendra Singh., Ahirwar, Narendra Kumar., Snehi, Sunil Kumar and Singh, V., 2015. Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbial and Biochemical Technology*, **7**(2):96–102.

Haas, Dieter and Defago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, **3**(4):307--319.

Haghighi, Barmak Jafari., Alizadeh, Omid and Firoozabadi, A.H., 2011. The role of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture. *Advances in Environmental Biology*, **5**(10):3079–3083.

Hayat, Rifat., Ali, Safdar., Amara, Ummay., Khalid, Rabia and Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology*, **60**(4):579--598.

Hayat, Rift, Ahmed, I., Sheirdil, Rabia and Amara., 2012. An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture, *M. Ashraf, ed., Springer Netherlands*, 557-559

Hol, WH Gera., Bezemer, T Martijn and Biere, A., 2013. Getting the ecology into interactions between plants and the plant growth-promoting bacterium *Pseudomonas fluorescence*. *Frontiers in plant science*, **4**(2):81.

Idris, A., Labuschagne, N and Korsten, L., 2009. Efficacy of rhizobacteria for growth promotion in sorghum under greenhouse conditions and selected modes of action studies. *The Journal of Agricultural Science*, **147**(1):17–30.

Kapulnik, Yoram., Okon, Yaacov., Kigel, Jaime., Nur, Israel and Henis, Y., 1981. Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by Setaria italica inoculated with Azospirillum brasilense (strain Cd). 340--343.

Karnwal, A., 2012. Screening of plant growth-promoting rhizobacteria from maize (*Zea mays*) and wheat (*Triticum aestivum*), **12(**3):6170–6185.

Kaur, H., Kaur, J., Gera, R., 2016. Plant Growth Promoting Rhizobacteria : A Boon to Agriculture. *International Journal of Cell Science and Biotechnology*, **5**:17–22.

Khalid, Arshad., Arshad, M and Zahir, Z., 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, **96**(3):473–480.

Khan, Mohammad Saghir., Zaidi, Almas and Musarrat, J., 2009. Microbial Strategies for Crop Improvement, *Springer Science and Business Media*, 105-124

Kloepper, JW., Schroth, MN and Miller, T. and others, 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield, 1078--1082.

Kochoni, Emeric., Didagbe, Yedeou O., Adjanohoun, Adolphe., Allagbe, Marcellin., Sikirou, Rachidatou., Gachomo, Emma W., Kotchoni, Simeon O., Baba-Moussa, L. and, 2013. Effect of Different Plant Growth Promoting Rhizobacteria on Maize Seed Germination and Seedling Development. *American Journal of Plant Sciences*, **4**(5):1013–1021.

Lemanceau, P and Alabouvette, C., 1993. Suppression of fusarium wilts by fluorescent pseudomonads: Mechanisms and applications. *Biocontrol Science and Technology*, **3**(3):219–234.

Luz, W.C. da, 2001. Evaluation of Plant Growth-Promoting and Bioprotecting Rhizobacteria on Wheat Crop. *Fitopatologia Brasileira*, **26**(3):597–600.

Mahdi, S Sheraz., Hassan, GI., Samoon, SA ., Rather, HA.,Dar, Showkat A and Zehra, B., 2010. Bio-fertilizers in organic agriculture. *Journal of phytology*, **2**(10):42–54.

Martinez-Viveros, O., Jorquera, MA., Crowley, DE., Gajardo, GMLM and Mora, M., 2010a. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of soil science and plant nutrition*, **10**(3):293–319.

Martinez-Viveros, O., Jorquera, MA., Crowley, DE., Gajardo, GMLM., Mora, M., 2010b. Mechanisms and practical considerations involved in plant growth promotion by

rhizobacteria. *Journal of soil science and plant nutrition*, **10**(3):293–319.

Mayak, S., Tirosh, T and Glick, B., 1999. Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. *Journal of Plant Growth Regulation*, **18**(2):49–53.

Mayak, S., Tirosh, T and Glick, B., 2001. Stimulation of the growth of tomato, pepper and mung bean plants by the plant growth-promoting bacterium Enterobacter cloacae CAL3. *Biological agriculture and horticulture*, **19**(3):261–274.

Menamo, Muluneh and Wolde, Z., 2015. Effect of Cyanobacteria Application as Biofertilizer on Growth, Yield and Yield Components of Romaine Lettuce (Lactuca sativaL.) on Soils of Ethiopia. *American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)*, **4**(1):50–58.

Nihorimbere, Venant., Ongena, Marc., Smargiassi, Maite and Thonart, P., 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnologie, Agronomie, Societe et Environnement*, **15**(2):327–337.

Pierson, Elizabeth A and Weller, D.M., 1994. Use of mixtures of flourescent pseudomonads to supress take-all and improve the growth of wheat. *Phytopathology*, **84**(9):940–947.

Podile, Appa Rao and Kishore, G.K., 2007. Plant growth-promoting rhizobacteria. *In: Gnanamanickam SS (ed) Plant-Associated Bacteria, Springer, Netherlands*, 195–230.

Pongdet, P., 2010. Effects of plant growth promoting rhizobacteria (PGPR) inoculum on indigenous microbial community structure under cropping system.

Praveen Kumar, G., Desai, S., Leo Daniel Amalraj, E., Mir Hassan Ahmed, SK and Reddy, G., 2012. Plant Growth Promoting Pseudomonas species from Diverse Agro-Ecosystems of India for Sorghum bicolor L . *Journal of Biofertilizers and Biopesticides*, **7**:2.

Raaijmakers, Jos M., Vlami, Maria and De Souza, J.T., 2002. Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**(1):537–547.

Reddy, Rajeshwar., Prasad, VR and Vemaraju, A., 2014. PGPR- A Potencial tool for sustainable agriculture: A review. *journal of Science*, **4**(2):117–122.

Saharan, B.S. and Nehra, V., 2011. Plant Growth Promoting Rhizobacteria : A Critical Review. *Life Sciences and Medicine Research*, **21**(1):30.

Schroth, Milton N and Hancock, J.G., 1981. Selected topics in biological control. *Annual Reviews in Microbiology*, **35**(1):453--476.

Sengupta, A. and Gunri, S.K., 2015. Microbial intervention in agriculture : An overview. *African Journal of Microbiology Research*, **9**(18):1215–1226.

Sharma, A. and Johri, B.N., 2003. Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS 9 in maize (*Zea mays L* .) under iron limiting conditions, 158:243–248.

Showkat, Sageera., Murtaza, Imtiyaz., Laila, Omi and Ali, A., 2012. Biological Control of Fusarium Oxysporum and Aspergillus Sp . By Pseudomonas Fluorescens Isolated From Wheat Rhizosphere Soil Of Kashmir . *Journal of Pharmacy and Biological Sciences*, **1**(4):24–32.

Shurti, Kaushal., Arun, Karnwal, Yuvneet, R., 2013. Potential Plant Growth-Promoting Activity of Rhizobacteria Pseudomonas sp in Oryza sativa. *J. Nat. Prod. Plant Resour*, **3**(4):38–50.

Silva, Krisle da., Perin, Liamara., Gomes, Maria de Lourdes., Barauna, Alexandre Cardoso., PEREIRA, Gilmara Maria Duarte., Mosqueira, Catia Aparecida., COSTA, Ismaele Breckenfeld da., O'HARA, Graham and Zilli, J.E., 2016. Diversity and capacity to promote maize growth of bacteria isolated from the Amazon region. *Acta Amazonica*, **46**(2):111–118.

Singh, M.P., 2015. Assessment of Antifungal Activity of PGPR (Plant Growth-Promoting Rhizobacterial) Isolates Against Rhizoctonia solani in Wheat (*Triticum aestivum L* .). *International Journal of Advanced Research*, **3**(10):803–812.

Sivasakthi, S., Usharani, G and Saranraj, P., 2014. Biocontrol potentiality of plant growth promoting bacteria (PGPR) - Pseudomonas fluorescens and Bacillus subtilis : A review. *African Journal of Agricultural Research*, **9**(16):1265–1277.

Stockwell, VO., Johnson, KB., Sugar, David and Loper, J., 2011. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. *Phytopathology*, **101**(1):113–123.

Sturz, AV and Christie, B., 2003. Beneficial microbial allelopathies in the root zone : the management of soil quality and plant disease with rhizobacteria. *Soil and Tillage Research*, **72**:107–123.

Suman, Biyyani, Gopal, A Vijara, Reddy, R.S. and T.. ., 2016. Isolation and Characterization of Pseudomonas fluorescens in the rice rhizospheric soils of Rangareddy district in Telangana state. *International Journal of Microbiology Research and Reviews*, **5**(1):164–169.

Suresh, A., Pallavi, P., Srinivas, P., Kumar, V Praveen., Reddy, S.R. and others, 2010. Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. *African Journal of Microbiology Research*, **4**(14):1491–1494.

Sureshbabu, K., Amaresan, N and Kumar, K., 2016. Amazing Multiple Function Properties of Plant Growth Promoting Rhizobacteria in the Rhizosphere Soil. *International Journal of Current Microbiology and Applied Sciences*, **5**(2):661–683.

Vacheron, Jordan., Desbrosses, Guilhem., Bouffaud, Marie-Lara., Touraine, Bruno and Prigent-Combaret, C., 2013. Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in plant science*, **4**:1–19.

Vejan, Pravin., Abdullah, Rosazlin., Khadiran, Tumirah., Ismail, Salmah and Nasrulhaq Boyce, A., 2016a. Role of Plant Growth Promoting Rhizobacteria in. *Molecules*, **21**(5):1–17.

Vejan, Pravin ., Abdullah, Rosazlin., Khadiran, Tumirah., Ismail, Salmah ., Nasrulhaq Boyce, A., 2016b. Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability—A Review. *Molecules*, **21**(5):1–17.

Wahyudi, A.T., Astuti, R.I. and Giyanto, 2011. Screening of Pseudomonas sp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. *American Journal of Agricultural and Biological Science*, **6**(1):134–141.

Walsh, Ultan F., Morrissey, John P and O'Gara, F., 2001. Pseudomonas for biocontrol of phytopathogens : from functional genomics to commercial exploitation. *Current Opinion in Biotechnology*, **12**(3):289–295.

Weller, David M., Raaijmakers, Jos M., Gardener, Brian B McSpadden and Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, **40**(1):309–348.

Weller, DM., Cook, R. and others, 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads, 463--469.

Weller, D.M., 2007. Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*, **97**(2):250–256.

Zehnder, Geoffrey W., Murphy, John F., Sikora, Edward J and Kloepper, J.W., 2001. Aplication of rhizobacteria for induced resistance. *European Journal of Plant Pathology*, **107**(1):39–50.

Zhuang, Xuliang., Chen, Jian., Shim, Hojae and Bai, Z., 2007. New advances in plant growth-promoting rhizobacteria for bioremediation. *Environment International*, **33**(3):406–413.

10. ANNEXES

Annex 1 Morphological and Microscopic characteristics of *Pseudomonas fluorescence* strains isolated on King's B medium

Cereal crops	Isolates	Colony characterization			Microscopic characterization		
		Size	Margin	Pigment	Gram reaction	Shape	Spore
Maize	P.f1	Large	Irregular	Yellowish green	Negative	Rods	Negative
Wheat	P.f2	Medium	Round	Yellowish green	Negative	Rods	Negative
Sorghum	P.f3	Small	Round	Yellowish green	Negative	Rods	Negative

Annex 2 Biochemical characteristics of Pseudomonas fluorescence species

Characteristics	Isolates		
	P.f1	P.f2	P.f3
Gram staining	–	–	–
Catalase	+	+	+
Urease test	–	–	–
Indole test	–	–	–
Methyl red test	–	–	–
Citrate utilization test	+	+	+
TSI test	+	+	+
Gelatin hydrolysis	–	–	–
Starch hydrolysis	–	–	–
Motility	Motile	Motile	Motile

Note: TSI =Triple sugar iron

+ Indicates positive results and - indicates negative results.

Declaration

We, the undersigned, declare that this scientific research paper is our own original work. All sources of materials, ideas and grant used for this research have been duly acknowledged.

Name of student with signature and date

Name of Advisor with signature and date